SUSTAINED RELEASE OF MICROCRYSTALLINE PEPTIDE SUSPENSIONS

BACKGROUND OF THE INVENTION

There is frequently a need to deliver biologically active peptides to animals and humans in formulations providing a sustained release of the active principle. Such formulations may be provided by incorporating the active principle in biodegradable and biocompatible polymers in form of microcapsules, microgranules or implantable rods, or alternatively using mechanical devices such as micropumps or non-biodegradable containers. If the peptide is highly soluble in aqueous media, it can be formulated as a complex with non-degradable polymers such as cellulose derivatives, or mixed with polymer solutions, which form a gel upon parenteral injection, from which the active peptide is slowly released.

All the above-mentioned formulations have drawbacks and limitations, such as the large volume of suspending fluids or the need to remove the non-degradable device. In the case of gel forming peptides, there is frequently a problem of bioavailability, which interferes with the desired sustained action of the active principle.

Some of the problems due to physico-chemical aspects of peptides have been described in article by R. Deghenghi "Antarelix" in Treatment with GnRH Analogs: Controversies and Perspectives", edited by M. Filicori and C. Flamigni, The Parthenon Publishing Group, New York and London 1996, pages 89-91. Additional problems were illustrated by J. Rivier "GnRH analogues towards the next millennium" in GnRH Analogues, edited by B. Lunenfeld, The Parthenon Publishing Group, New York and London 1999, pages 31-45 and by other workers such as M.F. Powell et al. "Parenteral Peptide Formulations: Chemical and Physical Properties of Native LHRH and Hydrophobic Analogues in Aqueous Solution" in Pharmaceutical Research, Vol. 8, 1258-1263 (1991).

Accordingly, there is a need for new formulations and methods of administration that avoid these problems, and this need is addressed by the present invention.

SUMMARY OF THE INVENTION

The invention relates to a method of preparing a sustained release formulation of a peptide or peptidomimetic. This method advantageously comprises associating or contacting the peptide or peptidomimetic with a counter-ion in an amount and at a molar ratio that are sufficient to provide a fluid, milky microcrystalline aqueous suspension without formation of a gel.

The invention also relates to a fluid, milky microcrystalline aqueous suspension of a peptide or peptidomimetic and a counter-ion in water, wherein the peptide and counter-ion are present in amounts and at a molar ratio sufficient to form, upon mixing, the suspension without formation of a gel.

The avoidance of a gel enables an injectable suspension to be formulated. When these aqueous suspensions are injected parenterally (i.e., subcutaneously or intramuscularly) into a mammal, such as a human, a sustained release of the peptide over time is obtained. Generally, this sustained release lasts at least about 2 weeks to one month or even to about 45 days or longer.

Preferably, the counter-ion is a salt of a strong proton donor. Most preferred counter-ions are strong acids such as trifluoro methanesulfonic acid, benzenesulfonic acid, trifluoroacetic acid or sulfuric acid. The peptide may be a somatostatin analogue, such as Vapreotide, Octreotide, Lanreotide or SOM-230. Also, the peptide may be a GnRH analogue, and preferably is a GnRH antagonist. More preferred GnRH antagonists include Azaline B, Abarelix, Antide, Ganirelix, Cetrorelix, or FE200486. These peptides are preferably present in the suspension in the form of their acid salts, e.g., sulfonate, trifluoroacetate or sulfate salts. Ac-

D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 trifluoroacetate and Ac-D-Nal-D-Cpa-D-Pal-Ser-Tyr-D-Hci-Leu-Ilys-Pro-D-Ala-NH_2 sulfate are the most preferred compounds.

The peptide or peptidomimetic preferably forms a salt with the counter-ion, and the salt is preferably suspended in the aqueous medium at a concentration of at least 25 mg/ml and has a molar ratio of at least 1.6:1 of counter-ion:peptide. The salt is at least partially in the form of microcrystals having a particle size of between about 1 and 150 μm.

If desired, the aqueous suspension can contain an isotonic agent, such as mannitol. Also, the aqueous suspension may contain a pharmaceutically acceptable excipient. Advantageously, the suspension is dried to a lyophilized state which can be reconstituted by mixing with water or a buffer solution. Lyophilized compositions comprising these dried suspensions, as well as the methods for making the dried suspensions and reconstituting them as aqueous suspensions, represent additional embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph which illustrates the pharmacodynamic effect (testosterone suppression) obtained by subcutaneous injection in rats of a suspension of Teverelix® trifluoroacetate according to the invention; and

Figure 2 is graph which illustrates the sustained release of the peptide Teverelix® for several weeks in rats injected with the suspension of Teverelix® trifluoroacetate according to the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to the unexpected discovery that certain peptides can be prepared or associated with various counter-ions and simply formulated to provide desirable suspensions of the peptide, which suspensions are

highly useful for administering the suspension by injection. In particular, a fluid, milky, stable microcrystalline suspension of the peptide is obtained without formation of a gel that would interfere with the handling of the suspension or the bioavailability of the peptide after injection.

The peptide that is to be utilized in the present suspension can be any one of a variety of well known bioactive peptides or peptide analogues which mimic such peptides. Advantageously, these peptides are formulated to obtain a delayed and sustained release of the peptide after injection. While any peptide can be utilized in this invention, those peptides or peptidomimetics having between 3 and 45 amino acids have been found to be the most suitable. In particular, representative peptides or peptidomimetics are well known to those of ordinary skill in the art and need not be exhaustively mentioned here. Typical examples include GnRH analogues and antagonists, as well as somatostatin and analogues thereof. Specific peptides include Azaline B, Abarelix, Antide, Ganirelix, Cetrorelix, FE 200486, Vapreotides, Octreotide, Lanreotide and SOM-230. These peptides have between 6 and 12 amino acids and are synthetically made to mimic the biological activity of GnRH or somatostatin. The examples mention further preferred peptides.

It has been found that certain counter-ions are highly preferred for obtaining sustained release of the peptide. Suitable counter-ions are those which are strong proton donors. While many compounds are well known to provide this function, the most preferred are strong acids. Sulfuric acid, a well known commodity, is quite useful for this purpose, as are other strong inorganic acids. Sulfuric is preferred due to its ready formation of suitable sulfate salts with the peptides of the invention. Strong organic acids can also be used as counter-ions. These acids include sulfonic acids, such as trifluoro methane sulfonic acid and benzene sulfonic acid. Others, such as trifluoro acetic acid or other fluorinated acids can be used if desired.

The amount of counter-ion is preferably that which is in excess of what is necessary to form a stoichiometric salt of the peptide. The amount of counter-ion is typically at least 1.6 mol acid/mole peptide and preferably 2mol/mol or greater. While no upper limit has been determined, the amount can be as high as 10 mol/mol. In addition, the injectable suspension should be concentrated to obtained the most desirable release profiles. By concentrated, we mean that the amount of peptide should be above 2.5% by weight of the overall formulation. This is conveniently achieved by adding to water or a buffer solution at least 25 mg/ml of the peptide. Amounts of as high as 100mg/ml can be used, and these suspensions can also contain other additives. In addition to conventional pharmaceutically acceptable excipients, an isotonic agent, such as mannitol, can be included for its known purpose. Other usual pharmaceutical additives can be included, as desired.

The suspensions can be dried by freeze-drying or spray drying to form lyophilized compositions that can be stored as is and later reconstituted with sterile water or buffer solutions when an injectable formulation is to be prepared. These lyophilized compositions can be stored for relatively long periods of time prior to use. Also they can be easily sterilized and handled until the time when they are to be reconstituted.

An additional advantage of this discovery is the small volume of such suspensions, allowing parenteral injections through a fine needle and thus improving the local tolerance of the injected material. Furthermore, the material can also be used for the local treatment of diseased tissues, e.g., brachytherapy. The peptide is partially or totally in the microcystalline form having a particle size of between about 1 and 150μm, and preferably between about 5 and 25μm. These small particles easily pass through the injection needle. In such injections, the amount of peptide ranges from about 0.1 to 5mg per kg body weight of the mammal or human to which the suspension is to be administered.

A specific discovery was that a highly concentrated aqueous suspension of the peptide of the formula Ac-D-Nal-D-pClPhe-D-Pal-Ser-Tyr-D-Hci-Leu-Lys(iPr)-Pro-D-Ala-NH_2 (Teverelix®, a GnRH antagonist) as a trifluoroacetate (TFA) or sulfate salt does not, as might be expected by its hydrophobic character, form a gel but instead forms a microcrystalline milky suspension which is easy to inject parenterally in animals or humans, and which releases the active principle over several weeks (see Figures 1 and 2). Such behavior is not elicited by other salts such as the acetate, which result in the expected, but unwanted, formation of gels with poor bioavailability *in vivo*.

The invention thus represents a simple and elegant solution to the problem of how to suppress gelation of peptide salts while obtaining a prolonged sustained delivery of peptides in the form of highly concentrated suspensions.

EXAMPLES

Example 1

200µL of 5% mannitol were added to approximately 15mg of the LHRH antagonist Teverelix® trifluoroacetate. The mixture was stirred using vortex during one minute and a flowing milky pearly suspension was obtained. The suspension is made of microcrystals of about 10µm length. Microcrystals may clump together to form urchin like structures. The suspension was injected in rats (1mg) sub-cutaneously and provided the pharmacodynamic effect of testosterone suppression for more than 45 days (Figure 1). The pharmacokinetic analysis showed a sustained release of the peptide for several weeks (Figure 2).

Example 2

200µL of water were added to approximately 15mg of the LHRH antagonist Teverelix® trifluoroacetate. The mixture was stirred using vortex during one minute and a flowing milky pearly suspension was obtained.

Example 3

200μL of water were added to approximately 15mg of the LHRH antagonist Teverelix® acetate. The mixture was stirred using vortex during one minute and a transparent gel was obtained. The addition of 20μL of TFA (3mols/mol) to the gel resulted in the formation of a fluid, flowing milky pearly suspension.

Example 4

200μL of 100mM TFA were added to approximately 15mg of the LHRH antagonist Teverelix® acetate (2mols/mol) to obtain a flowing milky suspension. In addition, mixing 200μL of 75mM TFA with approximately 15 mg of the LHRH antagonist teverelix® acetate (1.5mol/mol) resulted in a transparent gel being obtained after mixing. In another study, 100μL of TFA of various concentrations were added to 7.5mg of the LHRH antagonist teverelix® acetate, with the TFA/Teverelix molar ratio ranging from 1 to 3. A flowing milky suspension was obtained with molar ratios of 1.6, whereas gels were obtained at other molar ratios.

Example 5

200μL of 150mM TFA were added to amounts of the LHRH antagonist Teverelix® acetate ranging from 5 to 30mg (concentration ranging from 25 to 150mg/ml). A flowing milky suspension was obtained with concentrations up to 100mg/ml.

Example 6

200μL of 150mM TFA were added to approximately 15mg of the LHRH antagonist Teverelix® acetate (3mols/mol) and a flowing milky suspension was obtained after mixing. The suspension was freeze-dried over-night. 200μL of water or 5% mannitol were added to the lyophilisate and a flowing milky suspension was obtained after mixing and reconstitution.

Example 7

1mL of 150mM TFA were added to approximately 75 mg of the LHRH antagonist Teverelix® acetate (3mols/mol) and a flowing milky suspension was obtained after mixing. The suspension was freeze-dried over-night. 1mL of water and 0.2M acetate buffer pH 4.0 were added to the lyophilisate and a flowing milky suspension was obtained after mixing and reconstitution. These suspensions were stable for at least 3 days at room temperature.

Example 8

100μL of a 250mM H_2SO_4 were added to 7.5mg of the LHRH antagonist Teverelix® acetate (5mols/mol) and a flowing milky suspension was obtained after several hours. The suspension is made of microcrystals of about 100μm length. Microcrystals may assemble together to form urchin like structures. The suspension was freeze-dried over-night. 100μL of water or 5% mannitol were added to the lyophilisate and a flowing milky suspension was obtained after mixing and reconstitution.

Example 9

100μL of a 150mM trifluoromethane sulfonic acid solution were added to 7.5 mg of Teverelix ® acetate to obtain a free flowing milky suspension after mixing.

Example 10

100μL of a 150mM solution of benzenesulfonic acid were added to 7.5 mg Teverelix® hydrochloride to give after a mixing a free flowing suspension.

Example 11

100µL of a 200mM solution of trifluoroacetic acid solution were added to 2.5 mg of Cetrorelix® acetate to obtain a milky free flowing suspension.

Example 12

Free flowing suspensions were obtained by adding 100µL of a 150mM trifluoroacetic acid solution to 7.5 mg each of the following somatostatin analogues:

D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp-NH_2

D-2Me-Trp-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Trp(2Me)-NH_2

D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp(2Me)-NH_2

D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp(2Me)-NH_2

Example 13

100µL of a 5% mannitol - water solution were added to approximately 5 mg of the somatostatin analog known under the designation SOM 230, i.e., ETD-carboxy-c[Hyp-Phg-D-Trp-Lys-Tyr(Bzl)-Phe], as the trifluoroacetate salt. A milky free flowing suspension was thus obtained.